

# Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning

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## SUMMARY

1. Many aquatic ecosystems sustain multiple invasive species and interactions among them have important implications for ecosystem structure and functioning. Here, we examine interactions among two pairs of invasive crayfish species because of their close proximity and thus chance of sympatric populations in the near future within the Thames catchment, U.K. (signal, *Pacifastacus leniusculus* and virile crayfish, *Orconectes virilis* within a river system; red swamp, *Procambarus clarkii* and Turkish crayfish, *Astacus leptodactylus* found within a suite of ponds). We address two questions: do sympatric invasive crayfish occupy a smaller niche than their allopatric counterparts due to potential resource competition? and do interactions among invasive species amplify or mitigate one another's impacts on the ecosystem?
2. Two fully factorial mesocosm experiments (one for each crayfish pair) were used to investigate crayfish diet and their impact on benthic invertebrate community structure, benthic algal standing stock and leaf litter decomposition rates in allopatric and sympatric populations, compared with a crayfish-free control. We used stable isotope analysis to examine crayfish diet in the mesocosms and in allopatric populations of each species in the Thames catchment.
3. Isotopic niche width did not vary significantly between allopatric and sympatric populations of crayfish in the mesocosm experiments, and isotopic niche partitioning in all the wild populations suggests the invaders can coexist.
4. All four species altered benthic invertebrate community structure but with differing functional effects, often mediated via trophic cascades. Red swamp crayfish predation upon snails evidently promoted benthic algal standing stock via reduction in grazing pressure. However, a trophic cascade whereby the crayfish consumed native invertebrate shredders, causing a reduction in net leaf litter decomposition, was decoupled by red swamp and signal crayfish since they consumed leaf litter directly and thus moderated the cascade to a trickle when in sympatry with Turkish or virile crayfish, respectively.
5. Benthic invertebrate predator abundance was significantly reduced by sympatric red swamp and Turkish crayfish but not independently when in allopatry, indicating an amplified effect overall when in sympatry.
6. Our results suggest that the combined effect of multiple invasions on the ecosystem can reflect either an additive effect of their independent impacts or an amplified effect, which is greater than the sum of their independent impacts. A lack of general pattern in their effects makes any potential management strategy more complex.

**Keywords:** crayfish, ecosystem functioning, invasive species, stable isotopes, trophic cascades

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## Introduction

Biological invasions are recognised as a significant driver of global environmental change with consequences from the individual through to the whole ecosystem level of organisation (Sala *et al.*, 2000; Simon & Townsend, 2003; Ricciardi, 2007). Invasive species can severely disrupt the organisation of native communities by displacing native species or by reducing their abundance (Gurevitch & Padilla, 2004). These alterations in species assemblages and biodiversity can have major implications for ecosystem processes (Olden *et al.*, 2004; Hector & Bagchi, 2007; Pejchar & Mooney, 2009). As the pace of global change accelerates, many ecosystems sustain multiple invaders (e.g. Cohen & Carlton, 1998; Ricciardi, 2006; Jackson & Grey, 2013), and the interactions among them will have important consequences. In the U.K. alone, there are estimated to be almost 2000 non-native species, costing £1.7 billion in management costs and damage per annum (Roy *et al.*, 2012). With other environmental stressors such as climate change facilitating invasions (Rahel & Olden, 2008), many more invaders are on the horizon (Gallardo & Aldridge, 2013a,b).

Despite recent progress in invasion biology (e.g. Sanders *et al.*, 2003; Johnson *et al.*, 2009; Catford *et al.*, 2012), our knowledge regarding specific ecological impacts, and the mechanisms behind them, remains limited for most invaders. This is particularly true of animal invasions in aquatic habitats; there is a distinct bias in the literature towards studying terrestrial plant invasions (Lowry *et al.*, 2013). There is even less research on multi-species invasions involving complex interactions between invasive species and how those invaders integrate within food webs of native communities (Kuebbing, Nuñez & Simberloff, 2013).

Empirical evidence supports both facilitative and negative interactions between sympatric invaders. The invasional meltdown model predicts that disturbance caused by one invasive species will facilitate the establishment of further invaders (Simberloff & Von Holle, 1999; Simberloff, 2006) by, for example, removing a natural competitor or predator (Grosholz, 2005). Invasive species might act in synergy to magnify their independent impacts on ecosystem structure and functioning (Simberloff & Von Holle, 1999; Simberloff, 2006; Johnson *et al.*, 2009). On the contrary, there is the possibility that sympatric invasive species will moderate one another's impacts by each controlling the abundance of the other via competitive or predator–prey interactions (Lohrer & Whitlatch, 2002; Alonso & Martínez, 2006;

Griffen, Guy & Buck, 2008). The impact of coexisting invaders can also be independent; for example, invasive signal crayfish (*Pacifastacus leniusculus*) and rainbow trout (*Oncorhynchus mykiss*) in Sweden had independent impacts on prey, and hence, their combined effects on ecosystem structure were additive (Nyström *et al.*, 2001).

Decapods are extremely successful and disruptive invaders in many aquatic ecosystems (Karatayev *et al.*, 2009). Some crayfish species in particular are widespread, conspicuous invaders with a range of impacts on ecosystem structure and functioning (Capinha, Leung & Anastacio, 2011). They are omnivores and often the largest invertebrates within a food web; omnivory can decouple trophic cascades and alter energy flow, and hence, crayfish as invaders have a disproportional impact on food-web dynamics (e.g. Lodge *et al.*, 1994; Nyström, Brönmark & Granéli, 1999). Invasive species of crayfish regularly outcompete and replace native crayfish (Hill & Lodge, 1999; Alonso & Martínez, 2006; Dunn *et al.*, 2009; Haddaway *et al.*, 2012), but interactions among invasive crayfish are rarely examined despite the increasing likelihood of them occurring in sympatry. While competition has resulted in serial replacement of invasive crayfish in some instances (Hill & Lodge, 1999), coexisting populations are known (Nakata *et al.*, 2005; Bernardo *et al.*, 2011), but their combined interactive effect on the ecosystem is unknown. Furthermore, research focusing on the impact of invasive crayfish on biodiversity and ecosystem services is becoming increasingly important to inform management decisions as invasions become more widespread (Lodge *et al.*, 2012).

There are seven species of crayfish with established populations at the present time in the United Kingdom, only one of which is native (Holdich, Rogers & Reynolds, 1999; Keller, Zu Ermgassen & Aldridge, 2009). Four of the invaders occur in the Thames catchment (Jackson *et al.*, 2012) and yet little, if anything, is known of how they might interact. Invasive species can have negative implications for biodiversity, and the many valuable ecosystem services provided by freshwater environments (Pejchar & Mooney, 2009). Understanding how invaders are incorporated into communities, and unravelling how interactions among them impact upon the ecosystem, will underpin our understanding of the whole ecosystem impacts of multiple invasions and therefore be valuable in management and policy decisions (Lodge *et al.*, 2012).

Populations of signal and virile (or northern, *Orconectes virilis*) crayfish occur in separate reaches of the Lee Navigation canal, north-east London (Ahern, England &

Ellis, 2008), and separate populations of red swamp (*Procambarus clarkii*) and Turkish (*Astacus leptodactylus*) crayfish occur in adjacent ponds in Hampstead Heath, North London (Ellis *et al.*, 2012). At the time of study, all four species occurred in separate communities, with no range overlap. However, they have the potential to expand their range in the near future and, given the current close proximity of each crayfish pair in the two separate waterbodies (signal and virile in the Lee Navigation and red swamp and Turkish in Hampstead Heath), this could result in sympatric populations of these pairs in the Thames catchment. Therefore, we aim to predict what the outcome of these range expansions might be. We used field data and mesocosm experiments to examine potential and actual interactions between signal and virile crayfish, and between red swamp and Turkish crayfish, to address the question: do interactions among invaders amplify or mitigate one another's impact on ecosystem structure and functioning? We also used stable isotope analyses to examine dietary interactions between the invaders to answer the question: Do sympatric invasive species occupy a smaller niche than their allopatric counterparts due to potential resource competition?

## Methods

### Field survey

Using a combination of nitrogen and carbon stable isotope ratios ( $^{15}\text{N} : ^{14}\text{N}$  and  $^{13}\text{C} : ^{12}\text{C}$ ), it is possible to establish an animal's food sources and trophic level relative to an isotopic baseline (Post, 2002; Grey, 2006). We collected samples of all crayfish species for stable isotope analysis from allopatric populations in the Thames catchment using crayfish traps baited with fish pellets. Red swamp and Turkish crayfish were collected from ponds on Hampstead Heath in North London (the 'Bird Pond' and 'Hampstead 1', respectively). Signal crayfish and virile crayfish were collected from sites on the Lee Navigation in north-east London (Carthagen Lock in Broxbourne and Pickett's Lock in Edmonton, respectively). We sampled 8–15 individuals (Syvaranta *et al.*, 2013) from each population in the summer of 2009 and 2010. Muscle samples were dissected from the tail of each crayfish and oven-dried overnight at 60 °C to constant weight before analysis at Queen Mary, University of London, using an elemental analyser (Flash EA, 1112 series; Thermo-Finnigan) coupled to a continuous flow mass spectrometer (Finnigan MAT Delta<sup>Plus</sup>; Thermo-Finnigan, Bremen, Germany). Ratios of  $^{15}\text{N} : ^{14}\text{N}$  and

$^{13}\text{C} : ^{12}\text{C}$  are expressed in parts per mille (‰) using conventional delta notations ( $\delta$ ) relative to international standards (ammonium sulphate and sucrose, of known isotopic composition in relation to atmospheric nitrogen in air (N) and Pee Dee Belemnite (C); Ings, Hildrew & Grey, 2010).

Following Olsson *et al.* (2009), we corrected the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of the crayfish from the field sites, for trophic position (TP) based on the nitrogen values of long-lived baseline primary consumers (native bivalve molluscs from each site;  $n = 3\text{--}6$ ), and for  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}_{\text{corr}}$ ) against the carbon values of a representative sample of benthic invertebrates ( $n = 18\text{--}24$ ), respectively (see Appendix S1). This allowed us to confidently compare the trophic ecology of each crayfish species over spatial and temporal scales, regardless of variations in the isotopic baseline.

### Mesocosm experiment

Two outdoor mesocosm experiments, each of 42 days duration, were conducted during late summer and autumn 2010 using twenty fibreglass ponds (kidney-shaped ponds; 0.4 m deep; c. 1.5 m<sup>2</sup> benthic area). The first experiment examined interactions between signal and virile crayfish; the second, between red swamp and Turkish crayfish. Each mesocosm was prepared as follows: 2 cm depth of clean rock and gravel was added as a substrate and overlaid with 38 cm depth of rainwater which was continually aerated by aquaria pumps with air stones. A half-section of drainpipe (20 cm length) was added as a crayfish refuge, and a stack of three terracotta tiles (10 × 10 cm), each separated by 5 mm, was added as an invertebrate refuge. Macrophytes (1 *Mentha aquatica* and 1 *Callitriche* sp.) were planted in each mesocosm, and freshwater *Daphnia*, purchased from a local pet shop, were added in equal densities. Each mesocosm was then seeded with equal aliquots of kick samples of benthic invertebrates from the same sites from which the crayfish were sourced (Lee Navigation in experiment one; Hampstead Heath in experiment two) to mimic natural conditions and allowed to establish for one week before crayfish were added. Each experiment on each crayfish pair comprised five replicates of four treatments using a factorial design (i.e. crayfish A, crayfish B, crayfish A + B, no crayfish; Table 1). Each crayfish replicate had an equal density (four crayfish m<sup>-2</sup>; within the range of signal crayfish densities found in the wild in the U.K.; Guan, 2000; Bubb, Thom & Lucas, 2004) and biomass of crayfish of the same sex (Table 1).

**Table 1** The replicated ( $n = 5$ ) treatments used in each experiment

Experiment 1	Experiment 2
SC (6; $171 \pm 10$ g)	RSC (6; $224 \pm 10$ g)
VC (6; $171 \pm 10$ g)	TC (6; $224 \pm 10$ g)
SC (3; $85 \pm 5$ g) and VC (3; $85 \pm 5$ g)	RSC (3; $112 \pm 5$ g) and TC (3; $112 \pm 5$ g)
Neither species (0; 0)	Neither species (0; 0)

The numbers in parentheses represent the number of individual crayfish used from each species in each replicate followed by the total biomass of those individuals. Crayfish abbreviations are as follows: red swamp crayfish, RSC; Turkish crayfish, TC; signal crayfish, SC and virile crayfish, VC.

### Dietary analysis

On the final day of each experiment, crayfish were frozen overnight and then thawed before dissecting a portion of muscle from the tail for the analysis of stable isotopes. Samples of leaf litter, macrophytes and invertebrates from each pond were also taken for stable isotope analysis and prepared as above. We were confident that the duration of the experiment was sufficient for isotopic turnover based upon data from controlled feeding experiments on aquatic species, including crayfish, under similar temperature conditions (natural fluctuations between 5 and 17 °C; e.g. Bosley *et al.*, 2002; Gamboa-Delgado *et al.*, 2011; Carolan *et al.*, 2012). However, we also tested for isotopic differences between those individuals caught from survey sites and corresponding individuals from the same survey sites subjected to the 42 days in the mesocosms and checked for trajectory of change (Grey, Waldron & Hutchinson, 2004; Appendix S2).

### Ecosystem structure and functioning

Leaf litter of *Alnus glutinosa* was collected and dried before being used in the experiments to examine decomposition rates. Three plastic mesh bags (aperture 1, 5 and 10 mm) containing a known mass (*c.* 3 g) of the air-dried leaf litter were fastened to the bottom of each mesocosm at the start of the experiments. Only the 10-mm bags were accessible to crayfish; the 5-mm bags were accessible to other smaller benthic invertebrates, and the 1-mm bags were only accessible to microbial organisms. This allowed the direct and indirect impacts of crayfish on leaf litter breakdown to be quantified. The leaf litter remaining in each mesh bag after six weeks was washed and then dried at 60 °C to constant weight. The exponential decay rate coefficient ( $k$ ) was calculated for each treatment as a measure of

the rate of leaf litter decomposition following Hieber and Gessner (2002) as:

$$k = \frac{\log_e \left( \frac{M_t}{M_0} \right)}{t}$$

where  $t$  is the duration of exposure (in days),  $M_0$  is the initial dry mass (in grams) and  $M_t$  is the dry mass at time  $t$ .

We measured periphytic algal standing stock by placing a terracotta tile (5 × 5 cm) in each mesocosm at the start of the experiment. Tiles were removed on the final day of the experiment, and all biofilm was scrubbed, washed off and filtered through GF/C filters (Whatman®, Maidstone, U.K.) before adding 15 mL of 90% acetone. After 24 h in the dark, the samples were centrifuged and the supernatant was used for spectrophotometry. We then used chlorophyll-*a* concentration, quantified following Jeffrey and Humphrey (1975) and expressed as mg cm<sup>-2</sup> as a measure of periphyton standing stock.

We quantified the benthic invertebrates at the end of the experiment by removing, counting and identifying all organisms in 25 L of filtered water, two 15 cm<sup>2</sup> sediment cores, leaf packs and invertebrate refugia (three stacked tiles separated by 5 mm each). All invertebrates from each mesocosm were merged as one sample and stored in 70% IMS prior to identification and counting. After identification, benthic invertebrates were assigned to functional feeding groups for further analysis.

### Data analyses

To examine the trophic ecology of the crayfish from the field sites, we plotted standard ellipse areas (SEA<sub>c</sub>; Jackson *et al.*, 2011, 2012) based on the distribution of individuals (8–15 per sample) in isotopic space as an estimate of each species core trophic niche using the SIAR package (Parnell *et al.*, 2010; Jackson *et al.*, 2011) in the 'R' computing programme (R Core Development Team 2012). The subscript 'c' indicates that a small sample size correction factor was used (Jackson *et al.*, 2011). Past studies have indicated that a sample size of >5 is appropriate to reveal population niche width using SEA<sub>c</sub> (Jackson *et al.*, 2011).

Analysis of variance (ANOVA; Minitab 14®) was used to test for differences in δ<sup>13</sup>C and δ<sup>15</sup>N between populations of crayfish in each experiment. Discriminant analysis was used to investigate whether the species' classification could correctly predict the identity of each individual based on the stable isotope data.



We used the stable isotope-derived population metrics carbon range ( $CR_b$ ) and nitrogen range ( $NR_b$ ) as measures of the trophic niche width of crayfish from the mesocosm experiments (Layman *et al.*, 2007; Jackson *et al.*, 2011, 2012).  $CR_b$  and  $NR_b$  were calculated in the statistical package 'R' (R Core Development Team 2012) as the distance between the individuals with the highest and lowest  $\delta^{13}C$  and  $\delta^{15}N$  values, respectively. To enable comparison among variable sample sizes (three individuals in the treatments with two species of crayfish and six individuals in the treatments with allopatric crayfish), the metrics were bootstrapped ( $n = 10\,000$ ; indicated with a subscript 'b') based on the minimum sample size of three. ANOVA was then used to test for difference in  $CR_b$  and  $NR_b$  between populations of crayfish. We used permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) to test for effects of the experimental manipulations on the structure of benthic invertebrate assemblages using the PERMANOVA+ add-in to PRIMER<sup>®</sup>, version 6.1 (PRIMER-E Ltd, Plymouth, U.K.). These analyses were carried out with 9999 permutations of the residuals under a reduced model (Anderson, Gorley & Clarke, 2008) and were based on Bray–Curtis similarity matrices calculated from  $\log(X + 1)$ -transformed data. Similarity percentages analysis (SIMPER in PRIMER<sup>®</sup>; Clark & Warwick, 2001) was then used to determine the contribution of benthic invertebrate taxa to the mean Bray–Curtis dissimilarities between treatments using presence or absence of each crayfish species as factors. This method determines which taxa were affected most strongly by the presence of each species of crayfish in each experiment.

We used ANOVA to test for an effect of treatment on leaf litter breakdown, algal standing stock, benthic invertebrate richness and the total abundance of each benthic invertebrate functional feeding group. The Student–Newman–Keuls procedure was used following all ANOVA tests to make *post hoc* comparisons among levels of significant terms. All analyses were balanced, and variables were transformed where necessary prior to analysis to homogenise variances.

Finally, for clarity and to allow comparison between experiments, we calculated the standardised effect size of each treatment on each variable compared with the relevant experimental control, which had no crayfish. We used Hedges'  $d$  as a measure of effect size (Gurevitch, Morrison & Hedges, 2000; Vila *et al.*, 2011); in our analysis, the effect size estimates the standardised mean difference between each treatment and the control for each variable we quantified (invertebrate abundance and richness, periphyton standing stock and decomposition), and we used it to highlight similarities and discrepan-

cies in the effect of each invasive species on the ecosystem (Sanders *et al.*, 2003). For each mean response variable ( $X$ ), the individual effect size  $d$  of each crayfish treatment ( $i$ ) compared with the crayfish-free control ( $u$ ) was calculated as:

$$\frac{X_i - X_u}{S}j$$

where  $j$  is a weighting factor based on the number of replicates ( $n$ ) per treatment, calculated as:

$$1 - \frac{3}{4(n_i + n_u - 2) - 1}$$

and  $S$  is the pooled sample variation, calculated as:

$$\sqrt{\frac{(n_i - 1)\sigma_i^2 + (n_u - 1)\sigma_u^2}{n_i + n_u - 2}}$$

Finally, variance of  $d$  ( $Vd$ ) was calculated as:

$$\frac{n_i + n_u}{n_i n_u} + \frac{d^2}{2(n_i + n_u)}$$

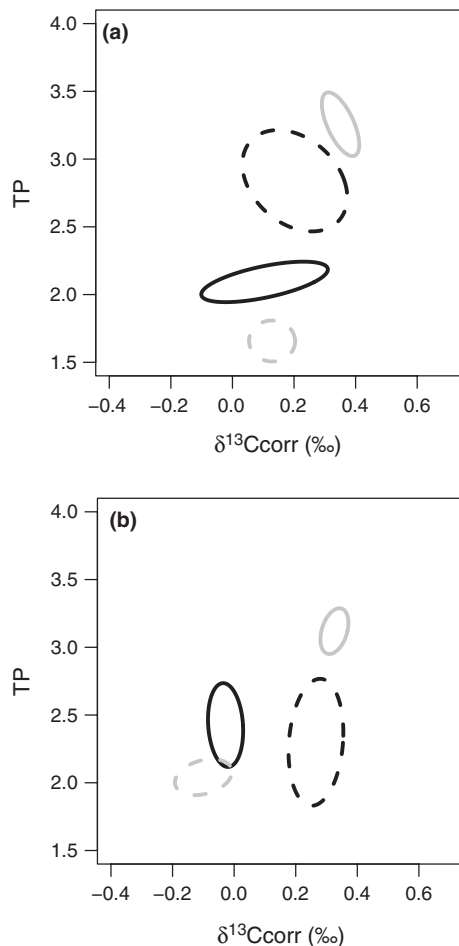
## Results

### Field survey

The core niche ( $SEA_c$ ) of each invasive crayfish population from the field sites did not overlap in isotopic space suggesting niche partitioning between species (Fig. 1). The field survey revealed that virile crayfish had the largest dietary niche in both years (2009:  $0.189\text{‰}^2$ ; 2010:  $0.129\text{‰}^2$ ) followed by signal (2009:  $0.076\text{‰}^2$ ; 2010:  $0.055\text{‰}^2$ ), Turkish (2009:  $0.036\text{‰}^2$ ; 2010:  $0.037\text{‰}^2$ ) and red swamp crayfish (2009:  $0.034\text{‰}^2$ ; 2010:  $0.022\text{‰}^2$ ; Fig. 1).

### Dietary interactions

In the mesocosms, the  $\delta^{15}N$  was significantly lower in populations of signal compared with virile crayfish ( $F_{3,80} = 31.93$ ,  $P < 0.001$ , Fig. 2a), and Turkish compared with red swamp crayfish ( $F_{3,77} = 42.07$ ,  $P < 0.001$ , Fig. 2b), suggesting a degree of niche partitioning across trophic levels. Sympatric virile crayfish also had a significantly higher  $\delta^{15}N$  than their allopatric counterparts ( $F_{3,80} = 31.93$ ,  $P < 0.001$ ; Fig. 2a). The  $\delta^{13}C$  was significantly lower in populations of allopatric signal crayfish compared with virile crayfish ( $F_{3,80} = 6.06$ ,  $P = 0.001$ ; Fig. 2a), and red swamp crayfish compared with Turkish crayfish ( $F_{3,77} = 19.76$ ,  $P < 0.001$ ; Fig. 2b), indicating niche partitioning across resources. Discrimination analysis correctly classified 88.1% of signal crayfish (37



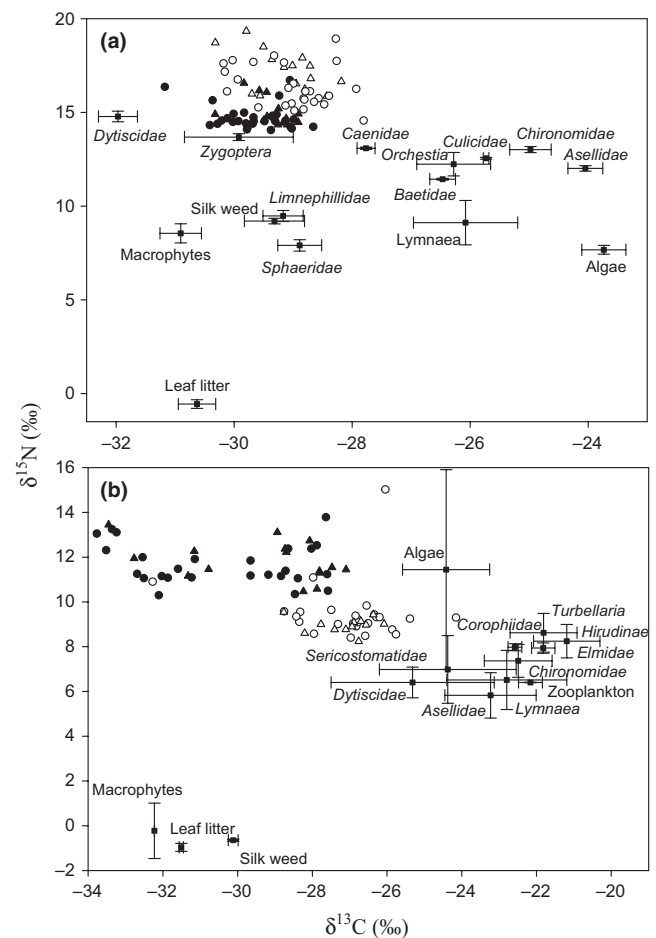
**Fig. 1** Isotopic biplot of trophic position (TP) and corrected  $\delta^{13}\text{C}$  values ( $\delta^{13}\text{C}_{\text{corr}}$ ). Each ellipse encloses the core niche width ( $\text{SEA}_C$ ) of signal (solid black), virile (dashed black), red swamp (solid grey) and Turkish (dashed grey) crayfish from the field sites in 2009 (a) and 2010 (b).

of 42), 83.3% of virile crayfish (35 of 42), 90.5% of red swamp crayfish (38 of 42) and 93.3% of Turkish crayfish (36 of 39), suggesting each species occupied a distinct area in isotopic space. All crayfish had changed isotopically during the experiment, assuming the field survey isotope values as a starting point, and in differing directions (Appendix S2).

Nitrogen and carbon range, both measures of isotopic niche width, were highest in virile and red swamp crayfish, respectively. However, niche width did not vary significantly between allopatric and sympatric populations of the same species (see Figure S1).

#### Assemblage composition

The benthic invertebrate community structure differed significantly between treatments in the signal and virile experiments ( $\text{Pseudo-}F_{3,16} = 2.30$ ,  $P = 0.01$ ). In both



**Fig. 2** Stable isotope biplot of the mesocosm food webs. Closed squares present the average isotopic signature of resources from all ponds (mean  $\pm$  standard error). (a) Experiment 1; filled symbols represent individual signal crayfish, and open symbols represent individual virile crayfish in allopatric (circles) and sympatric (triangles) populations. (b) Experiment 2; filled symbols represent individual red swamp crayfish, and open symbols represent individual Turkish crayfish in allopatric (circles) and sympatric (triangles) populations.

experiments, the assemblage was the most similar in treatments with crayfish (average similarity between 65.2% and 76.6%); therefore, the control treatments were the most distinct. The same five taxa were affected the most by signal or virile crayfish and, in total, they contributed almost 50% to the dissimilarity in the community assemblage (Table 2). Variation in just three taxa explained more than 50% of the dissimilarity in the community assemblage between both red swamp and Turkish crayfish absence or presence (Table 3).

The total abundance of grazers and scrapers was reduced in all crayfish treatments compared with the controls (experiment 1:  $F_{3,16} = 2.72$ ,  $P = 0.079$ ; experiment 2:  $F_{3,16} = 4.75$ ,  $P = 0.015$ ; Figures S2 and S3); the effect size was largest in treatments containing Turkish and red

**Table 2** Results of SIMPER analysis indicating the primary benthic invertebrate taxa affected by the presence of signal (SC) and virile crayfish (VC)

Taxon	Mean abundance in SC absence	Mean abundance in SC presence	Contribution to dissimilarity (%)	Cumulative contribution (%)
Chironomidae	664 ± 125.0	193 ± 26.9	10.0	10
Limnephilidae	4.9 ± 1.1	12.5 ± 4.8	9.0	19.0
Corophiidae	10.1 ± 1.8	14.1 ± 4.2	8.8	27.8
Lymnaeidae	7.8 ± 3.2	3.0 ± 0.6	7.7	35.4
Gammaridae	5.0 ± 1.3	7.1 ± 2.7	7.47	42.9

Taxon	Mean abundance in VC absence	Mean abundance in VC presence	Contribution to dissimilarity (%)	Cumulative contribution (%)
Corophiidae	543 ± 149.0	314 ± 57.8	12.2	12.2
Limnephilidae	9.6 ± 3.6	7.0 ± 3.5	9.4	21.6
Gammaridae	16.0 ± 3.7	6.3 ± 1.3	9.0	30.6
Lymnaeidae	7.3 ± 3.2	5.8 ± 1.0	7.4	38.0
Chironomidae	7.7 ± 2.5	3.5 ± 0.6	6.5	44.5

Mean relative abundance ( $\pm$  standard error) is displayed using the raw data for clarity; however, the analysis was performed on  $\log(x + 1)$ -transformed data. Please note, standard errors will be high since each mean covers two treatments; for instance, abundances in SC absence will be an average of the neither species treatment and the VC-only treatment.

**Table 3** Results of SIMPER analysis indicating the primary benthic invertebrate taxa affected by the presence of red swamp (RSC) and Turkish crayfish (TC)

Taxon	Mean abundance in RSC absence	Mean abundance in RSC presence	Contribution to dissimilarity (%)	Cumulative contribution (%)
Lymnaeidae	62.0 ± 90.7	11.7 ± 13.3	20.1	20.1
Chironomidae	157 ± 95.4	196 ± 106.1	19.8	39.9
Asellidae	17.6 ± 21.7	14.5 ± 14.3	11.9	51.7
Turbellaria	9.9 ± 13.0	2.8 ± 6.1	9.3	61.0
Oligochaeta	2.8 ± 4.21	1.6 ± 1.2	5.4	66.4

Taxon	Mean abundance in TC absence	Mean abundance in TC presence	Contribution to dissimilarity (%)	Cumulative contribution (%)
Chironomidae	175 ± 121.7	166 ± 79.9	20.6	20.6
Lymnaeidae	64.2 ± 89.8	11.0 ± 9.3	20.2	40.1
Asellidae	19.6 ± 22.2	15.5 ± 12.7	12.1	52.9
Turbellaria	10.2 ± 13.23	3.7 ± 5.2	9.7	62.5
Dytiscidae	2.1 ± 2.7	2.5 ± 3.5	5.3	67.8

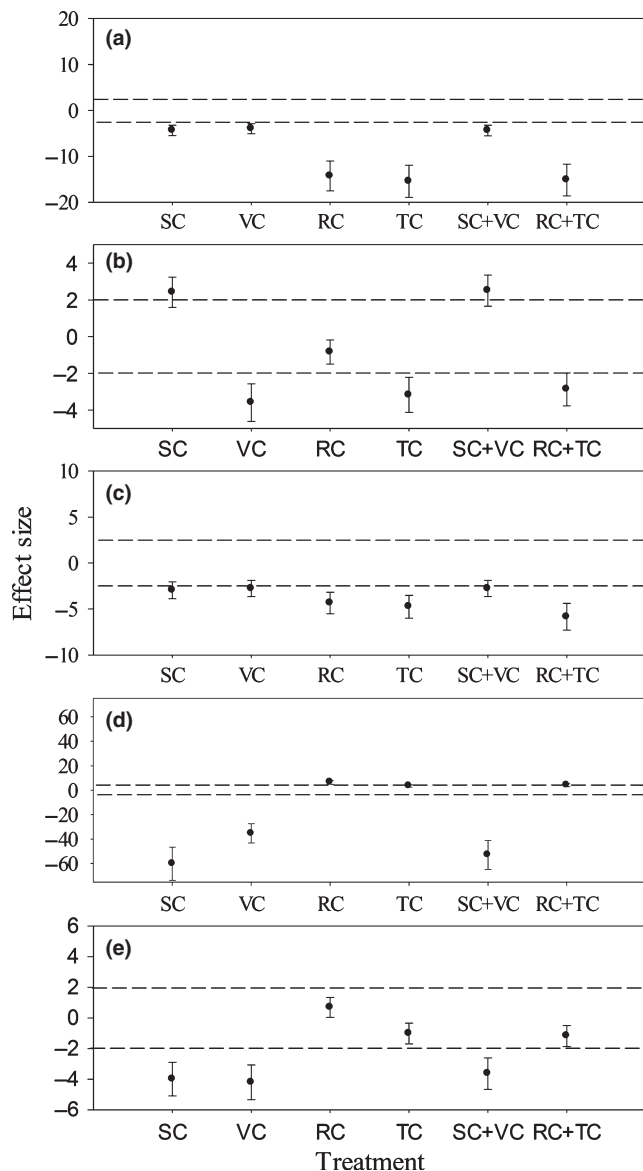
Mean relative abundance ( $\pm$  standard error) is displayed using the raw data for clarity; however, the analysis was performed on  $\log(x + 1)$ -transformed data. Please note, standard errors will be high since each mean covers two treatments; for instance, abundances in RSC absence will be an average of the neither species treatment and the TC-only treatment.

swamp crayfish (Fig. 3a). Abundance of gatherers and shredders did not differ significantly between treatments in both experiments (Figures S2 and S3); however, the largest effect we recorded was a decline in abundance in the allopatric Turkish and virile treatments (Fig. 3b). The largest effect on predator and carnivorous scavenger abundance was in the sympatric red swamp and Turkish crayfish treatments (Fig. 3C), where abundance was significantly lower compared with the control ( $F_{3,16} = 3.06$ ,  $P = 0.05$ ; Figure S3). Signal and virile crayfish treatments also had significantly lower numbers of predators and carnivorous scavengers ( $F_{3,16} = 8.78$ ,  $P = 0.001$ ; Figure S2,

Fig. 3c) and Diptera ( $F_{3,16} = 8.78$ ,  $P = 0.001$ ; Figure S2, Fig. 3d) compared with the control. Finally, benthic invertebrate taxon richness was highest in the treatment with no crayfish compared with all virile and signal crayfish treatments ( $F_{3,16} = 7.43$ ,  $P = 0.002$ , Fig. 3e).

#### Algal standing stock

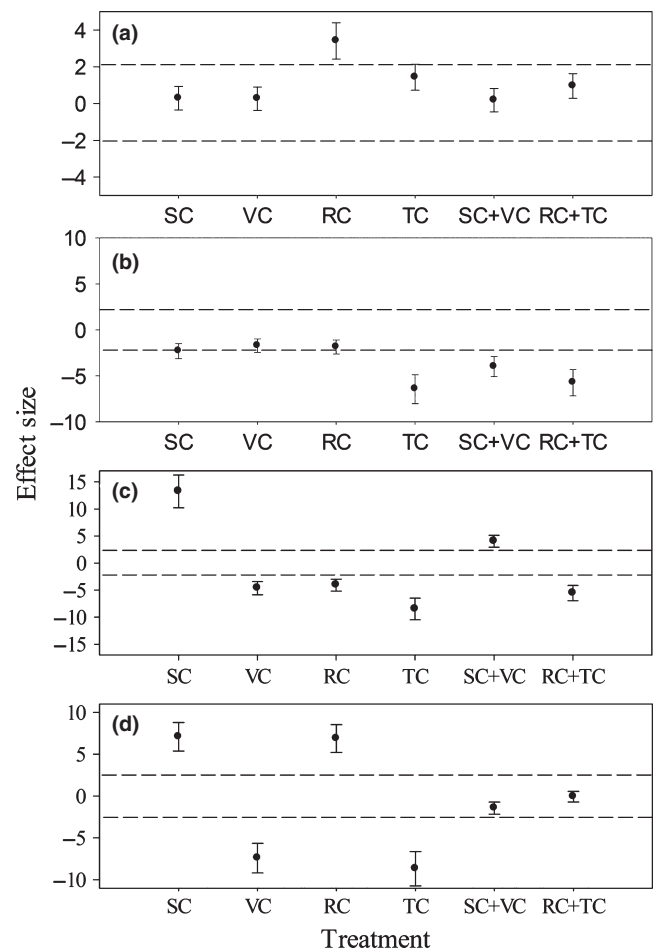
Periphyton standing stock was significantly higher in the treatment with allopatric red swamp crayfish compared with the control ( $F_{3,16} = 4.5$ ,  $P = 0.018$ ; Fig. 4a, Figure S4).



**Fig. 3** The effect size (Hedges'  $d$ ) of each variable in each treatment compared with the experimental control which had no crayfish. A positive  $d$  indicates an increase; negative  $d$  a decrease, in abundance or species richness relative to controls. Effects are statistically significant ( $P < 0.05$ ) if confidence limits do not overlap. Gatherers and shredders (a), grazers and scrapers (b), predators and carnivorous scavengers (c), Diptera (d), taxon richness (e).

#### Leaf litter decomposition

Microbial leaf litter decomposition was reduced in all crayfish treatments compared with controls (Fig. 4b); however, the effect was only significant in both treatments with Turkish crayfish present ( $F_{3,16} = 6.86$ ,  $P = 0.003$ ; Figure S5). In bags with a mesh aperture of 10 mm, which allowed access by crayfish and other invertebrates, leaf litter decomposition rates were



**Fig. 4** The effect size (Hedges'  $d$ ) of each variable in each treatment compared with the experimental control which had no crayfish. A positive  $d$  indicates an increase; negative  $d$  a decrease, in periphyton standing stock or decomposition relative to controls. Effects are statistically significant ( $P < 0.05$ ) if confidence limits do not overlap. Periphyton standing stock (a), microbial decomposition (b), benthic invertebrate decomposition (c), crayfish decomposition (d).

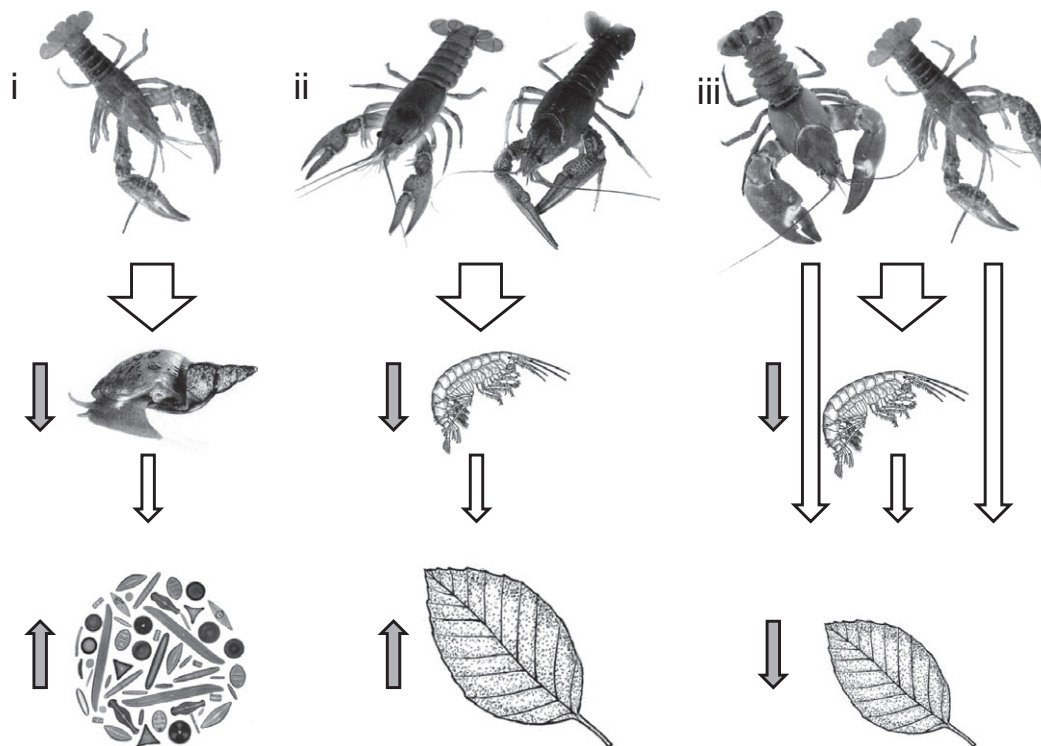
reduced in the allopatric virile and Turkish treatments and elevated in the allopatric signal and red swamp treatments (Fig. 4d). This effect was significant in the allopatric virile treatment compared with the control and allopatric signal treatments ( $F_{3,16} = 16.95$ ,  $P < 0.001$ ; Figure S5), and in the allopatric Turkish treatment compared with the allopatric red swamp treatment ( $F_{3,16} = 3.68$ ,  $P = 0.034$ ; Figure S5). Leaf litter decomposition by smaller invertebrates (i.e. in 5-mm aperture bags not accessible to crayfish) was reduced in all treatments bar those with signal crayfish present (Fig. 4c). In these bags, decomposition rates were significantly lower in the control and virile crayfish treatments compared with the allopatric signal treatment ( $F_{3,16} = 9.24$ ,  $P = 0.001$ ; Figure S5); and in the allopatric Turkish treatment compared with the control ( $F_{3,16} = 3.75$ ,  $P = 0.033$ ; Figure S5).



## Discussion

Our results demonstrate that multispecies crayfish invasions have different ecosystem level impacts compared with single-species invasions. Despite having generalist and omnivorous diets, we found dietary niche partitioning between each crayfish species, resulting in differences in their independent impacts on ecosystem structure and functioning. Niche partitioning suggests that interspecific competition will be low and therefore the niche width should be consistent between allopatric and sympatric populations (Gilbert, Srivastava & Kirby, 2008; Levine & HilleRisLambers, 2009). Accordingly, we found no evidence that crayfish niche width was reduced in the presence of a potential competitor. However, virile crayfish had significantly higher  $\delta^{15}\text{N}$ , reflecting a higher trophic level, in the presence of signal crayfish, indicating that competitive interactions may have caused a shift in their diet.

These differences in diet preference expressed by each invasive crayfish species, consequently affected ecosystem functioning through trophic cascades to differing degrees (Carpenter *et al.*, 1987). Similarly, Rudnick and Resh (2005) found that differences in the diet of invasive red swamp crayfish and Chinese mitten crabs (*Eriocheir sinensis*) resulted in variation in their impacts on the ecosystem. We found that slight diet variation between crayfish species triggered opposing impacts on the ecosystem in both autochthonous- and allochthonous-based food chains. Red swamp crayfish had the largest negative effect on invertebrate grazer and scraper abundance (particularly on snails from the genus *Lymnaea*) and subsequently were the only species to promote periphyton standing stock (see schematic in Fig. 5). Such trophic cascades through the autochthonous-based food chain involving a reduction in grazing pressure (Charlebois & Lamberti, 1996; Nyström *et al.*, 1999) may have ramifications for whole ecosystem functioning and alter primary



**Fig. 5** A schematic diagram illustrating the trophic cascades instigated by invasive crayfish in the experimental mesocosms. White arrows depict the direction of the cascade, and grey arrows indicate the effect of the trophic cascade on biomass compared with the control with no crayfish. Crayfish caused a decline in grazer abundance and therefore reduced grazing pressure on algae. In the red swamp crayfish treatments, this resulted in elevated benthic chlorophyll-*a* concentrations compared with the control (i). The crayfish also consumed invertebrates that shred and consume leaf litter; this impact was most evident in the virile and Turkish crayfish treatments and resulted in a decline in net decomposition rates (i.e. an increase in leaf litter biomass; ii). However, because crayfish are omnivores and will also consume leaf litter directly, they often decouple this trophic cascade resulting in elevated leaf litter loss and reduced leaf litter biomass; this effect was apparent in the signal and red swamp crayfish treatments (iii).

productivity (Carpenter *et al.*, 1987; Morin, Lamoureux & Busnarda, 1999; Jones & Sayer, 2003).

Crayfish simultaneously affect intermediate consumers and their basal resources because they are omnivores (Usio, 2000). In our experiments, all four invasive species independently altered decomposition rates by two opposing mechanisms, causing variation in allochthonous leaf litter availability (see schematic in Fig. 5). The three invertebrate taxa most affected by virile crayfish were all shredders or gatherers. In fact, both virile and Turkish crayfish had the greatest negative effect on invertebrate gatherer and shredder abundance which, via a trophic cascade, reduced leaf litter decomposition. In stark contrast, red swamp and signal crayfish promoted leaf litter decomposition by consuming leaf litter directly (reflected in their  $\delta^{13}\text{C}$  values more closely associated with leaf litter) in preference to associated invertebrate prey, which decoupled the trophic cascade. Since Turkish and virile crayfish had an opposite effect on net decomposition rates to red swamp and signal crayfish, we observed an intermediate effect on net decomposition rates in the sympatric treatments (multi-species invasion scenarios), which were indistinguishable from the controls. This suggests that the impact of multispecies crayfish invasions will be the sum of their independent impacts. Therefore, if two sympatric invasive species have similar independent impacts on the ecosystem (i.e. red swamp and signal crayfish both elevate decomposition rates), their combined effect might be additive. For example, invasive rusty crayfish (*O. rusticus*) and Chinese mystery snails (*Bellamya chinensis*) both independently reduce native snail biomass by consumption and competitive interactions, respectively, eliminating one species of native snail in the presence of both invaders (Johnson *et al.*, 2009).

There was also evidence of an amplified outcome of interactions among invaders. Invertebrate predator and carnivorous scavenger abundance was reduced in the presence of signal and/or virile crayfish, consistent with an *in situ* experiment by Stenroth and Nyström (2003) on signal crayfish in Sweden. However, red swamp and Turkish crayfish had no independent effect and, instead, only reduced predator and carnivorous scavenger abundance in multispecies invasion scenarios. This indicates an amplified impact whereby the presence of both species synergistically increased competition (McCarthy *et al.*, 2006) and/or predator–prey links (Stenroth & Nyström, 2003) with native invertebrate predators resulting in the largest observed negative effect on their abundance. There was also variation in the impact of invasive crayfish species on Dipteran larvae; abundance

was reduced in the presence of signal and virile crayfish only, suggesting direct consumption had a larger effect than the positive effect of sediment sorting by crayfish (Usio & Townsend, 2004). Finally, only virile and signal crayfish treatments had lower taxon richness than the treatments with no crayfish, consistent with other studies on signal crayfish (e.g. Stenroth & Nyström, 2003).

Lodge *et al.* (2012) recently made a call for more research on the impact of crayfish invasions; here, we have shown how multi-species crayfish invaders interact to impact ecosystem structure and functioning. Some variation in the independent effects of invasive crayfish had implications for their combined synergistic impact, resulting in amplified, intermediate and additive effects on the ecosystem. Our results have serious implications for conservation of biodiversity and management of invasive species by indicating that multiple crayfish species are able to coexist due to resource partitioning, which could promote higher densities in sympatric populations (Siepielski *et al.*, 2011). Crayfish density is positively correlated with the magnitude of its impact on ecosystem structure and functioning (Parkyn, Rabeni & Collier, 1997), and therefore, we predict that multispecies crayfish invasions will have an amplified effect on the ecosystem as a result of higher total crayfish densities.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** The nitrogen and carbon range of allopatric and sympatric crayfish populations in the mesocosms.

**Figure S2.** The total abundances of each functional feeding group in each treatment in experiment 1.

**Figure S3.** The total abundances of each functional feeding group in each treatment in experiment 2.

**Figure S4.** Algal standing stock in each treatment in both experiments.

**Figure S5.** Decomposition rates in each treatment in both experiments.

**Appendix S1.** Detailed methods on the corrections used to ensure field isotope data were comparable.

**Appendix S2.** Explanation of the evidence indicating that the crayfish used in the mesocosms had reached isotopic equilibrium.

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